Soil Acidity Tolerance of Symbiotic and Nitrogen-Fertilized Soybeans

D. N. Munns, J. S. Hohenberg, T. L. Righetti, and D. J. Lauter

ABSTRACT

In soybean (Glycine max L.), unlike other legumes, poor growth in acid soil might not be due to nodulation failure. This possibility was tested by observing time effects on nodulation, early growth, and N concentrations in symbiotic (N-dependent) plants as compared with control plants fertilized with NH₄NO₃. There were two lime × N factorial trials with acid soils: a greenhouse trial in a Typic Haploxeralf with two cultivars and 13 single-strain rhizobia inoculants; and an outdoor container trial in a Typic Hapludult, with nine cultivars and three rates of inoculation that produced large differences in nodule weight and number. The soils were high in exchangeable and soluble Al. In both trials, liming from pH 4.4 to 6.0 (aqueous paste) doubled growth, regardless of N-source, cultivar, or Rhizobium strain or numbers; and inoculated plants were nodulated, green, and high in N even when their growth was severely acid-affected.

Symptomatic indications that soybean growth in the acid soils was limited by Al toxicity to the host plant were confirmed in solution culture experiments with pH, Al, and Ca controlled at levels resembling those found in extracts of the soil solutions. Growth was unaffected by low Ca (200 μM) or low pH (4.5), but was depressed at 50 and 65 μM Al.

The data suggest that efforts to improve acid tolerance in soybean should center on plants, not rhizobia.

Additional index words: Legume nutrition, Rhizobium, Root nodulation, Nitrogen fixation, Aluminum, Calcium, Strain and cultivar selection.

IMPAIRMENT of legume nodulation and nodule function by an environmental stress can induce N starvation and limit growth of the host plant. Conversely, impairment of host plant growth by an environmental stress can limit nodulation and N₂ fixation (8, 10). The distinction between these two cases is still ignored at the detriment of otherwise useful research (3, 4). Yet it is a simple distinction to make. In both cases there will be less plant growth, nodulation, N yield, and acetylene reduction. But if slow N₂ fixation is limiting growth there will also be evidence of N-deficiency; e.g., low N, yellow leaves, and positive response to fertilizer N. Nitrogen-fertilized control plants will be less affected than symbiotic plants if the stress primarily impairs fixation.

Soil acidity has long been known to induce N-deficiency in legumes by preventing normal nodulation. Acidity, Al toxicity, and Ca deficiency inhibit Rhizobium growth, root infection, and nodule activity enough to account for symbiotic failure. This information agrees with more direct evidence of N deficiency, to support the general conclusion that legumes become more sensitive to soil acidity if they depend on symbiotic N₂ fixation (9). But the conclusion might not be correct for all legumes.

An important exception might be soybean (Glycine max L. Merr.). This paper presents evidence that soil acidity can impair growth of soybeans equally, whether they are N-fertilized or "symbiotic" (dependent on N₂ fixation), because the effective limitation of growth and N₂ fixation is the host's susceptibility to Al toxicity. The evidence came from simple experiments comparing responses of N-supplied and symbiotic plants to acidity in two soils, with 13 strains of Rhizobium japonicum and nine currently commercial varieties of soybean. The importance of Al was clarified by solution culture experiments comparing the factors pH, Al, and Ca controlled at levels representing those measured in the soil solutions.

MATERIALS AND METHODS

Soybean seeds were supplied by Dr. B. H. Beard from the USDA soybean breeding project at Davis. The varieties were 'Ada', 'Ansley', 'Clark', 'Evans', 'Grande', 'Kanrich', 'Lee', PI297590, and 'Williams'. They represent maturity groups 00, I, III, IV, and V.

Strains of Rhizobium were the most recently tested on soybean by the University of Hawaii NiFTAL Project (strains CBI809, CC709, USDA110, TAL102, and the O. N. Allen strains 511, 519, 527, and 542) and by the Nitragin Co., Milwaukee (6IA76, 61A101, 61A144, and 61A150). The inoculants were maintained on slants of yeast mannitol agar. Inoculants were made from yeast mannitol liquid cultures (barely turbid at 5 × 10⁹ cells/ml), by diluting with 0.3 M CaCl₂ + 0.3 M MgSO₄. Th dlutions were pipetted onto seed at planting immediately before covering, and subsamples were plate-counted.

Soils and soil treatments are outlined in Table 1. We used subsols from two California Ultisols chosen for ability to give large response to lime, N, and inoculation. Soil A, Josephine series (Typic Haploxerult), came from the foothills of the Sierra Nevada east of Sacramento. Soil B, Hugo-Josephine intergrade (Typic Haploxeralf), came from the Coast Range west of Sacramento. Both soils had kaolinite and oxide clay mineralogy, less than 0.1% organic matter, and no rhizobia capable of nodulating soybean. All treatments received 1 millimole of K₂SO₄/kg soil, and either 4 millimole KH₂PO₄ (Soil A) or 4 millimole Ca(H₂PO₄)₂ (Soil B). Zinc and Mo were supplied at 5 and 0.1 ppm, respectively. These basal fertilizer treatments and the pH amendments (Table 1) were mixed with the soil, followed by watering and a reaction period of 3 weeks (Experiment 1) or 15 weeks (Experiment 2).

Experiment 1

Growth at three pH levels was compared in Soil A. At each pH there were 15 single-strain inoculant treatments (each providing 5 to 9 × 10⁹ cells/seed) and uninoculated controls without N and with NH₄NO₃ (200 mg N/pot). Triplicate pots containing 2 kg soil were sown with soybean varieties Evans and Williams; and were thinned to two plants of each variety. pots were set in randomized blocks in large thermostat water baths, keeping soil temperature in the range 25 to 27 °C. Greenhouse air temperature ranged diurnally between 22 and 32 °C. Distilled water was added by weight to field capacity when required. At 42 days from sowing, plants were washed out of the soil; and shoot dry weight, nodule number, nodule weight, and percent N in shoots were measured.

Experiment 2

Growth of nine soybean varieties was compared at three pH levels in Soil B (Table 1). At each pH there was an NH₄NO₃ treatment (+N) and three rates of rhizobial inoculant. The NH₄NO₃, a single application 8 days after emergence, supplied 1.1 g N/plant. The inoculants were suspensions con-

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2 Professor of soil science and graduate research assistants.
Experiment 2 responded alike to soil pH. Strain treatments are pH (2, 7, 13). Inoculated plants at pH 4.4 still had nodules, whether plants were symbiotic or N-supplied versus plants subjected to low pH.

Table 1. Soils and soil treatments.

<table>
<thead>
<tr>
<th>Soil and treatment</th>
<th>pH (paste)</th>
<th>Exchangeable</th>
<th>Al in 0.01 M HCl, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Josephine soil</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11 millimol Al(SO₄)₂/kg</td>
<td>4.4</td>
<td>21.6</td>
<td>3.5</td>
</tr>
<tr>
<td>None</td>
<td>4.8</td>
<td>20.3</td>
<td>3.4</td>
</tr>
<tr>
<td>5 millimol CaCO₃/kg</td>
<td>6.2</td>
<td>32.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Hugo soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 millimol Al(SO₄)₂/kg</td>
<td>4.5</td>
<td>62.1</td>
<td>4.8</td>
</tr>
<tr>
<td>None</td>
<td>5.0</td>
<td>55.1</td>
<td>5.8</td>
</tr>
<tr>
<td>18 millimol dolomite/kg</td>
<td>6.5</td>
<td>84.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>

† Cations extracted with 1 M NH₄Cl at 30:1 solution ratio.  
‡ Extracted with a 10:1 solution to soil ratio.

The separate and combined effects of pH, Al, and Ca were tested on N-supplied Clark soybean in solution cultures. The levels of each variable (Fig. 3 and Table 2) were chosen to cover the range estimated to occur in soil solution in Trials 1 and 2. Nitrogen was supplied since normally effective nodulation is difficult to achieve with soybean in solution culture and the previous experiments had already indicated that symbiotic dependence would have little effect on sensitivity to the test variables. The solutions had low P to allow maintenance of Al in solution (7) and to resemble soil solutions in this important respect. To help control of P, pH, and Al, containers were large, 20 liters. The solution, constantly aerated, contained MgSO₄, NH₄NO₃, and KNO₃, each 1 mM; either CaCl₂ 1 mM or NaCl 0.2 mM with KCl 1.6 mM; KH₂PO₄ 20 µM in 3a and 10 µM in 3b, FeEDTA 10 µM, FeSO₄ 5 µM, H₂SO₄ 5 µM, MnSO₄ 1 µM, CaSO₄ 0.2 µM, ZnSO₄ 0.8 µM, NaNO₃ 0.2 µM, FeCl₃ 0.01 µM. Adjustments of pH with KOH or H₂SO₄ and of P concentration were made daily, if necessary. Aluminum was added as KAl(SO₄)₂ and checked weekly by analysis. Seeds were germinated on paper rolls wet with 0.2 M CaCl₂ and transplanted at 4 days, two plants (with four plants of Vigna unguiculata as part of another experiment) to each triplicate pot. Plants were harvested at 25 days (Experiment 3a) or 22 days (Experiment 3b) for determination of shoot weight, root weight, and taproot length.

RESULTS

Experiment 1

Regardless of soil pH, uninoculated control plants were yellow, sparsely nodulated, stunted and low in N; inoculated plants were nodulated, green, and high in N. Some rhizobia were slightly more effective than others, but in all rhizobial treatments the plants responded alike to soil pH. Strain treatments are pooled in Fig. 1 for comparison with +N at different pH.

Soil acidity inhibited soybean growth to the same degree, whether plants were symbiotic or N-supplied (Fig. 1). At pH 4.4, leaves were slightly darker green than normal, and roots were stubby as in Al toxicity (2, 7, 13). Inoculated plants at pH 4.4 still had nodules and high N.

Experiment 2

The nine varieties behaved alike (Fig. 2a). Data for varieties are pooled in Fig. 2b, 2c, 2d for presentation of effects of inoculant rate and soil pH. These factors interacted strongly on nodule number and nodule weight (Fig. 2c, 2d); i.e., low pH and low inoculum enhanced each other's adverse effect. This strong interaction was not reflected in plant growth. Acidity affected plant growth as in Experiment 1, regardless of inoculant treatment, plant variety, or the supply of N (Fig. 2a, 2b).

Experiment 3a and 3b

Aluminum reduced shoot weight, root weight, and root length, regardless of Ca concentrations up to 1 mM (Fig. 3), and produced symptoms like those observed in plants grown in soil at pH 4.4. Root length, but not root weight, was low at 0.2 mM Ca. Neither pH nor Ca affected shoot weight (Table 2).

DISCUSSION

Soil acidity responses on two different soils were tested with nine currently used North American cultivars, three rates of inoculation, and 13 highly effective Rhizobium strains. None of these variables made much difference to the plants' growth response.

Poor soybean growth at low soil pH was not due to N starvation. Affected plants remained green; their leaf N concentrations were high; N fertilization did nothing to alter the response to acidity. Nodulation at low pH was sufficient to supply the N-demand of the stunted plants despite some reduction in nodule number. Failure of the soybean cultivars in these acid soils can be attributed simply to Al sensitivity of the host plant. The growth depression and the symptoms (2, 7, 13) were reproduced in N-supplied plants subjected to the appropriate Al concentration in solution culture.

If soybean can nodulate effectively in acid soil where its growth is severely limited by Al toxicity, then soybean is an exception to the generality that legumes fail in acid soil because of poor nodulation. One consequence is that it becomes justifiable to ignore rhizobia and supply N when screening for acidsoil tolerance in soybean (2, 13). The same simplifi-
Fig. 1. Growth, nodulation, and percent N in soybean plants in Josephine soil at different pH (Experiment 1). Interactions involving plant variety and Rhizobium strain were not significant. Points represent pooled data for two varieties (Evans and Williams). Curves labeled "symbiotic" represent pooled data from 13 separate inoculation treatments.

Fig. 2. Effects of soil acidity on growth and nodulation in Hugo-Josephine soil (Experiment 2). No interactions involving cultivar were significant. Accordingly, data for cultivars are pooled except in Fig. 2a. Soil pH interacted highly significantly with N/inoculant treatments on nodule number (Fig. 2c) and weight (Fig. 2d), but not on plant growth (Fig. 2b). (Cultivar numbers 1-8 in Fig. 2a represent respectively 'Ada', 'Amsoy', 'Clark', 'Evans', 'Grande', 'Kanzich', 'Lee', PI1297550, and 'Williams'. Inoculant treatments R1, R2, and R3 supplied respectively $2 \times 10^5$, $2 \times 10^6$, and $2 \times 10^7$ cells/seed.)
cation is unjustified for other legumes. Nor may it be entirely justified for soybean, unless observations like ours can be generalized for different soybean types, *Rhizobium* strains, and soils.

Our findings are consistent with field trials in which large yield depressions due to acidity were evidently not accompanied by symptoms of N-deficiency (11, 14). Our data agree in detail with those of Sartain and Kamprath (12, 13) from experiments with high-Al Ultisols from North Carolina, including their observation that although nodule number correlated negatively with Al level, it was still high at the lowest soil pH (4.3 to 4.6), and percent N in shoots was unaffected.

Nevertheless, there is evidence that soil acidity sometimes causes nodulation failure and N starvation of soybean (1, 6). The experiments that show this may have involved less acid-tolerant rhizobia, very low inoculum levels, more Al-tolerant host varieties, or a type of acid-soil stress different from the Al-toxic condition imposed by Sartain and Kamprath and us. Indeed, in one case (1) the data suggest extreme Ca deficiency as the major stress component; in the other case (6) the soils were Histosols, extremely acid (pH 5.8 to 4.2) and low in Al. This might imply that results like ours should be expected only in the large group of acid soils where Al toxicity is the dominant limitation.

Soybean's ability to nodulate despite Al toxicity at pH 4.4 seems inconsistent with evidence that soybean rhizobia cannot grow well under these conditions (5). Possibly the seedlings sufficiently neutralized the rhizosphere soil to protect the rhizobia from acidity and Al. Some preliminary evidence supports this hypothesis (Munns and Vonich, unpublished).

There may be other legume-*Rhizobium* symbioses in which host growth rather than the symbiotic system is critically sensitive to soil acidity. In particular, such cases should become frequent if selection of acid-tolerant rhizobia is successful.

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**LITERATURE CITED**


